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The goal of the work supported by this grant is to characterize the molecular basis of prostate specificity and androgen independence of the *hoxb-13* gene with a view towards developing new treatments for advanced prostate cancer. To date, we have succeeded in generating a physical map of both the mouse and human hoxb-13 loci, and have sublconed regions both upstream and downstream of the coding region of both genes. Two reporter gene constructs carrying 4 or 8 kb of the human *hoxb-13* upstream region have been completed and transgenic mice carrying these constructs have been generated. Analyses of reporter gene expression in these mice have revealed that the 8 kb fragment directs a pattern of gene expression that recapitulates features of *hoxb-13* expression but does not direct expression in the prostate gland. Transgenes carrying regions farther upstream as well as downstream of *hoxb-13* loci are being constructed to widen the search for cis-acting elements that function in the prostate.

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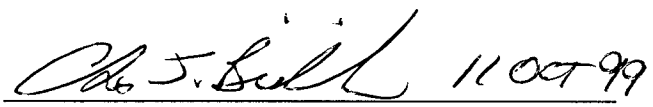

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INTRODUCTION

The goal of the work supported by this grant is to characterize the molecular basis of prostate specificity and androgen independence of the *Hoxb-13* gene with a view towards developing new treatments for advanced prostate cancer. Reporter gene analyses in transgenic mice will be used to identify the *cis*-acting region of the human *Hoxb-13* locus that confers this unusual pattern of gene expression. Deletion analyses will be employed to delineate the minimal functional *cis*-regulatory element. In vivo footprinting in cultured human prostate cancer cells will be used to characterize potential binding sites of transcriptional regulatory proteins. The identification of a prostate-specific, androgen-independent regulatory element would provide a unique reagent that could be incorporated into gene therapy strategies to treat advanced prostate cancer in patients that have undergone androgen deprivation therapy. Currently available prostate-specific regulatory elements are all androgen dependent and would be unlikely to function well in these patients. The in vivo footprint analyses may lead to the identification of new proteins or specific transcriptional complexes that can serve as potential targets for the development of therapeutics to disrupt gene expression in prostate cancer cells.

BODY- RESEARCH ACCOMPLISHMENTS

Task 1: To identify a region of the human *Hoxb-13* locus that is capable of directing expression of a reporter gene to prostatic epithelial cells in an androgen-independent manner (months 1-30).

Restriction mapping of hoxb-13 loci: The first experimental objective relevant to this task was to develop a physical map of the human *Hoxb-13* genomic locus. To this end, two pairs of PCR primers were designed based on the sequence of *Hoxb-13* available in Genbank accession number HSU 57052. Conditions for amplification of 192 or 175 base pair products from human genomic DNA were then optimized. Following optimization, one pair of primers was sent to Genome Systems, Inc. to be used for screening a human PAC library for clones containing *Hoxb-13* sequences. This screen succeeded in identifying a single PAC clone containing an insert estimated to be 200 kb in size.

Based on the partial sequence of *Hoxb-13* available in Genbank, a battery of restriction enzymes were chosen to digest the PAC clone in preparation for Southern blot analyses (Figures 1 and 2). This Southern filter was sequentially hybridized with two probes derived from the extreme 5' and 3' ends of the known *Hoxb-13* sequence. Molecular weight analyses of the components that hybridized with each probe allowed us to derive a physical map covering approximately 50 kb surrounding the *Hoxb-13* coding region. Restriction sites as far as 30 kb upstream and 20 kb downstream of the coding region were identified (Figure 3 and data not shown).

Southern blot analysis of HOXB-13 containing PAc clone with HOXB-13 5' RACE probe

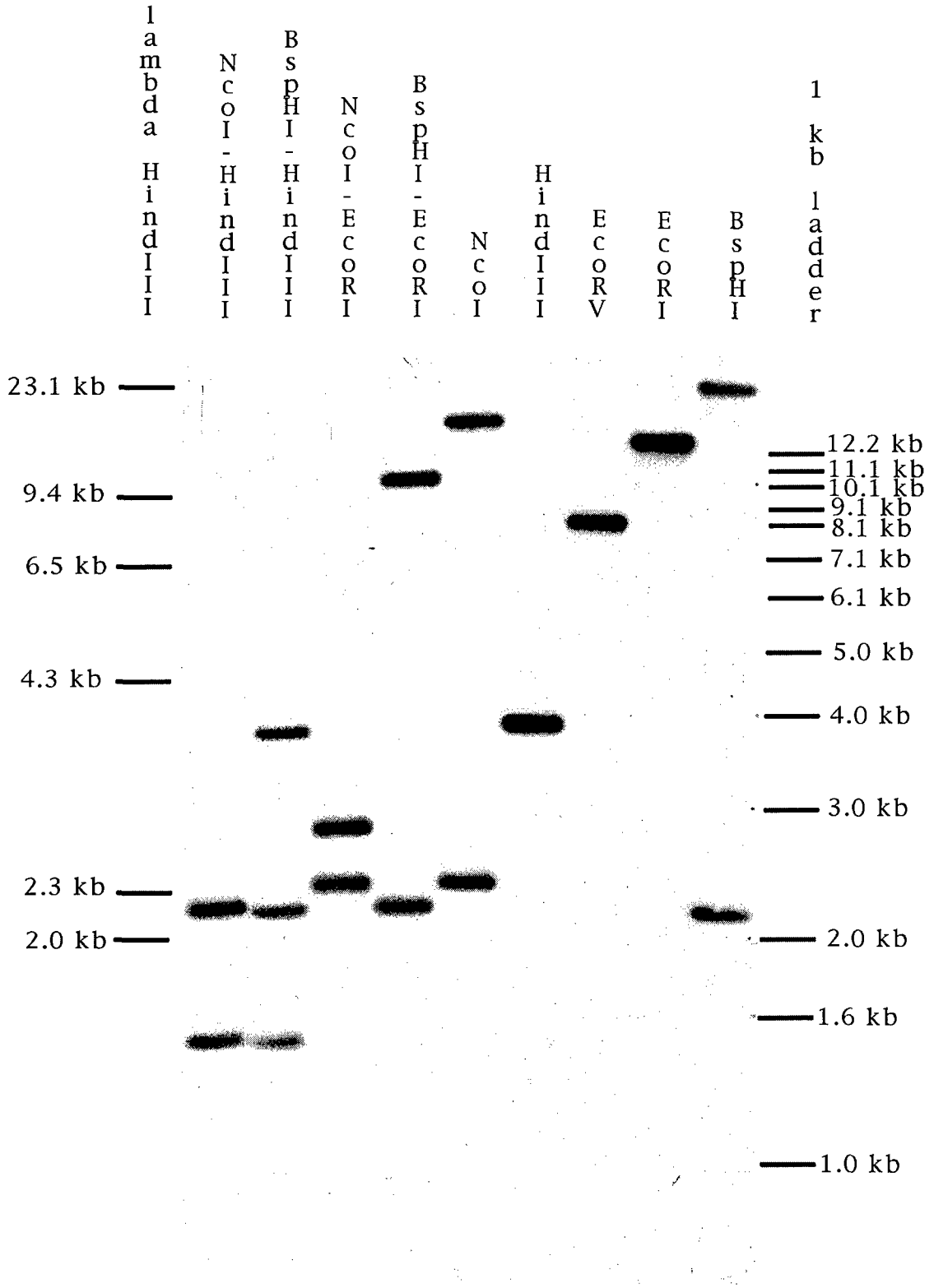

Figure 1 Southern blot analysis of Hoxb-13-containing PAC clone with a hoxb-13 5' probe. PAC clone was digested with the indicated restriction enzymes and hybrizided with a probe from the 5' end of the known Hoxb-13 sequence.

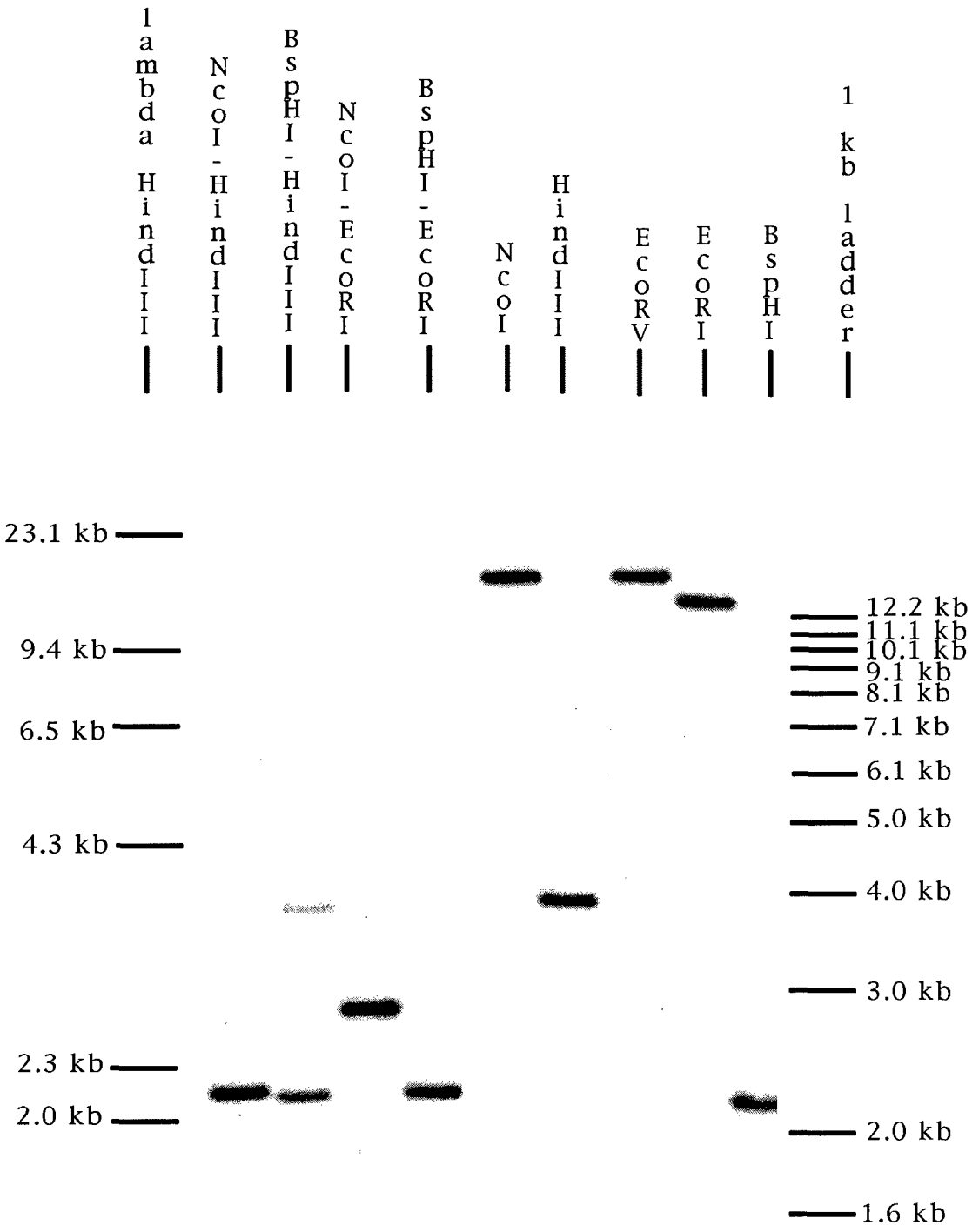

Figure 2 Southern blot analysis of Hoxb-13-containing PAC clone with a hoxb-13 3' probe. PAC clone was digested with the indicated restriction enzymes and hybrizides with a probe from the 3' end of the known Hoxb-13 sequence.

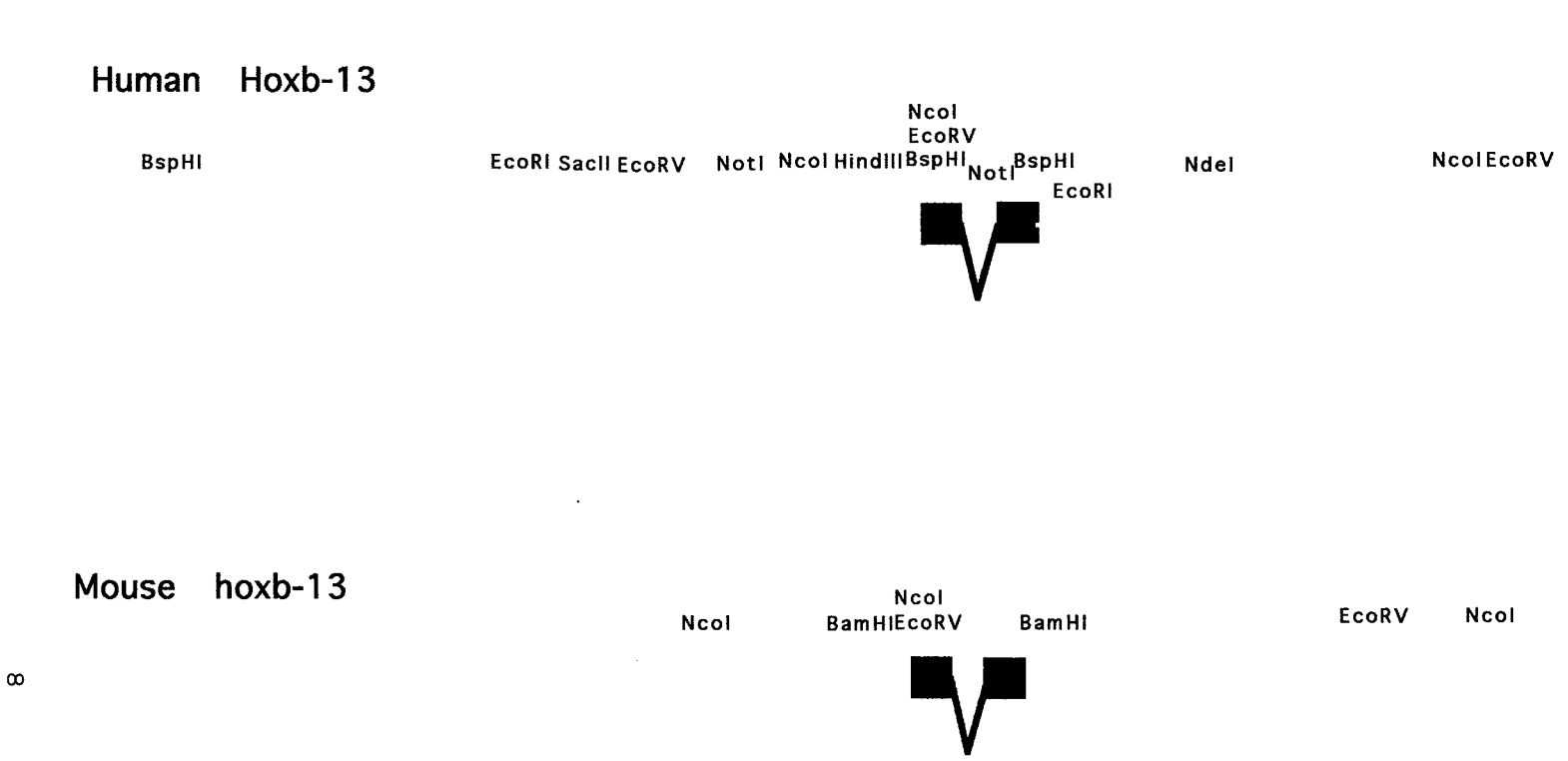

Figure 3 Partial restriction maps of human and mouse hoxb-13 loci. Restriction maps were generated based on Southern blot analyses using 5' and 3' clones from either mouse or human hoxb-13 genes. Dashed lines connect the indicated subcloned fragments to the map.

In parallel, we have also generated a physical map of the mouse *hoxb-13* locus. Although this goal does not appear in the approved Statement of Work, Specific Aim 1 includes a statement that mouse *hoxb-13* cis-acting regions may also be characterized. Using a Southern blot strategy similar to that described above for human *Hoxb-13*, a restriction map for the *hoxb-13* locus was generated using a *hoxb-13*-containing cosmid that had been previously identified (CJB, unpublished) (Figure 3).

Characterization of the transcriptional start site of hoxb-13: The transcriptional start site of the mouse *hoxb-13* has been identified using a 5' RACE strategy. RNA was extracted from mouse prostate glands. Several primers predicted to be within the coding region of *hoxb-13* and just downstream from the translational start site were designed and used to generate cDNA. cDNAs were then tailed using terminal deoxynucleotidyl transferase, PCR amplified, cloned, and sequenced. Comparison of the sequence obtained from the 5' RACE clone to the genomic sequence revealed that the transcriptional start site lies 135 bases upstream of the translational start site. Although the mouse and human *hoxb-13* genomic sequences are identical in this region, and we are confident that the human start site will be in the same location, 5' RACE analyses using RNA extracted from cultured human prostate epithelial cells will be performed.

Subcloning of human and mouse genomic fragments: In preparation for construction of reporter genes carrying putative cis-regulatory regions of *hoxb-13* loci, we have subcloned multiple fragments containing both upstream and downstream regions of *hoxb-13*. Although several of the fragments were readily cloned into conventional plasmid vectors, others proved to be refractory to cloning using standard technologies. Multiple attempts were made to clone these difficult fragments into pBluescript in a series of different bacterial hosts without success. We have recently overcome these difficulties by employing a non-conventional vector, pACYC184. For reasons that are not clear, but which may include the fact that pACYC184 is a low copy plasmid, all of the refractory-to-cloning fragments have now successfully been propagated in this vector.

Generation of initial reporter gene constructs: As outlined in the grant application, our initial strategy has been to survey the region immediately upstream of the *Hoxb-13* transcription unit for cis-regulatory elements capable of directing expression in the prostate. Two constructs have been completed: one contains a 4 kb fragment extending from the start of translation to a Not I site in the upstream region; the other extends an additional 4 kb in the upstream direction (Figure 3). In both of these constructs, the *Hoxb-13* sequences are cloned upstream of the bacterial lacZ gene.

Generation of transgenic mice carrying initial reporter gene constructs: Restriction enzyme fragments containing either 4 kb or 8 kb of human *Hoxb-13* upstream

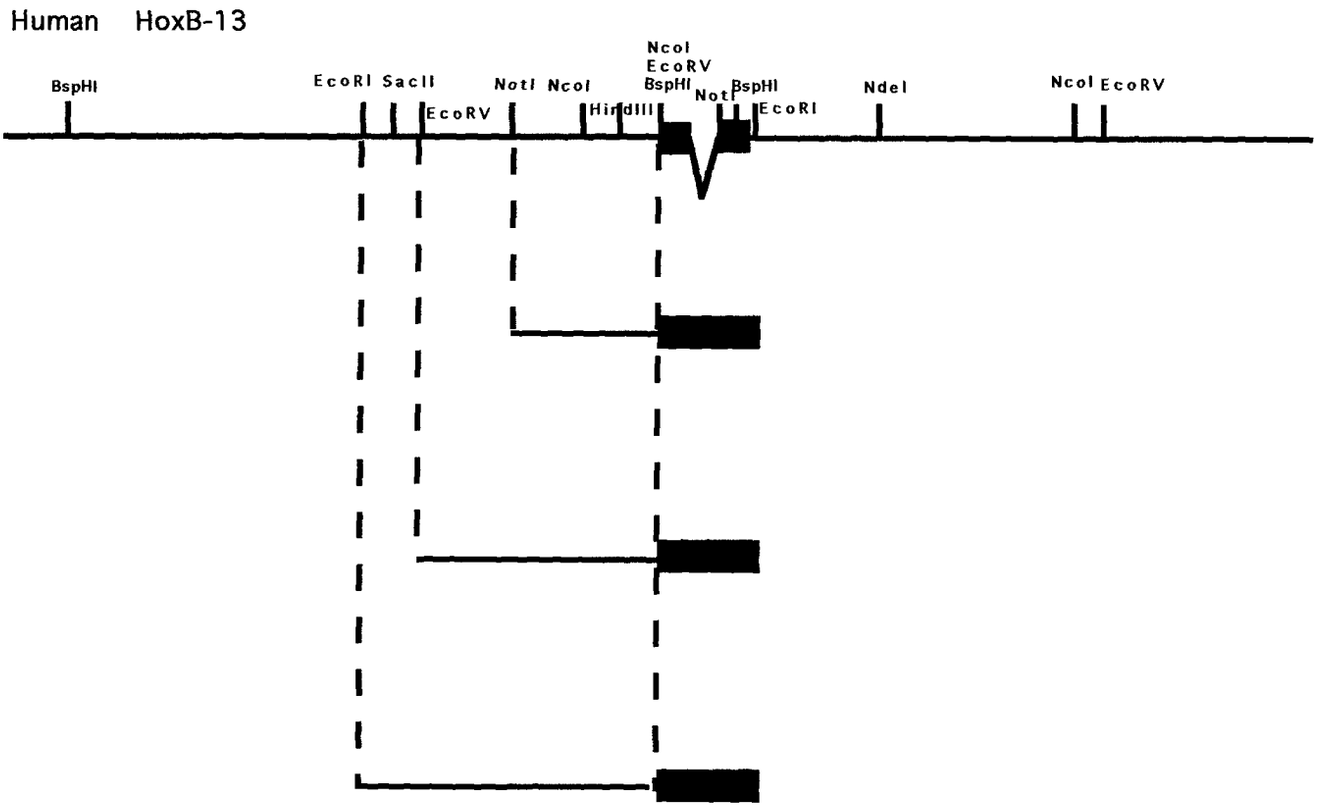

Figure 4 Human Hoxb-13-lacZ reporter gene constructs. The upstream fragments indicated by the dashed lines were cloned into a lacZ containing vector in preparation for generation of transgenic mice.

sequences directing expression of the lacZ reporter were isolated free of vector DNA by sucrose gradient fractionation and prepared for pronuclear injection into fertilized mouse eggs. The 4kb construct was injected into 562 eggs, 492 of which survived and were implanted into foster mothers. Twenty-five pups were born to these foster mothers. The 8 kb construct was injected into 280 eggs, 259 of which survived and gave rise to 21 pups.

Southern blot analyses of transgenic animals: To determine which mice had incorporated the reporter gene constructs into their genome, Southern blot analyses of DNA extracted from tail biopsies were performed. Genomic DNA was digested with EcoRV and the Southern filter was hybridized with a probe derived from the 5' end of human *hoxb-13*. EcoRV cuts once within the transgene and should yield a unit length transgene assuming multiple copies have integrated in a head-to-tail array, which is the typical configuration for most transgene integration events. This probe cross-hybridizes with a cognate fragment in the mouse genome, providing an internal reference for a single copy gene (Figure 4). Transgenic mice were identified as those mice displaying additional hybridizing components, the most prominent being a band of the molecular weight predicted for either the 4 or 8 kb upstream constructs. Four independent transgenic founder mice carrying the 4 kb construct were identified (data not shown) and six independent founders carrying the 8 kb construct were identified (Figure 4).

Derivation of F_1 generation transgenic animals and analyses of reporter gene expression: Transgenic founder mice were bred to normal FVB mice to derive an F1 generation. Southern blot analyses were performed to identify transgenic F1s. To determine whether the reporter gene was expressed in a pattern that reflected the endogenous *Hoxb-13* gene, transgenic F1 embryos and adult tissues (including prostate) were examined for lacZ activity using a standard in situ whole mount X-gal staining protocol. None of the transgenic offspring from founders carrying the 4 kb construct showed any lacZ activity at embryonic stages or in adult tissues. In contrast, transgenic offspring from founders carrying the 8 kb construct did display prominent regions of lacZ activity. In embryos, expression was detected at 10.5 days gestation in a very posterior domain in the neural tube and presomitic mesoderm. This pattern fits well with the predicted pattern of *hoxb-13* expression at this stage of development (Figure 6). By 10.5 days gestation, lacZ activity was also detected in the myotome portion of developing somites. This site of expression is not predicted based on the published pattern of *hoxb-13* expression, and may reflect the presence of control regions from neighboring genes present within the 8 kb construct. At 12.5 days gestation, the endogenous *hoxb-13* gene initiates expression in the urogenital sinus in the future prostatic region. Unfortunately, the transgenic mice did not display lacZ activity in the urogenital sinus or its derivatives at 12.5 days or in subsequent stages, including adult tissues (Figure 7 and data not shown). These data suggest that the 8 kb construct contains elements that are capable of recapitulating most of the features of embryonic *hoxb-13* expression with the exception of urogenital, and eventually,

prostate expression. We are currently driving constructs that carry additional upstream and downstream *hoxb-13* sequences to search for the cis elements that direct prostate expression in accordance with the strategy outlined in the proposal.

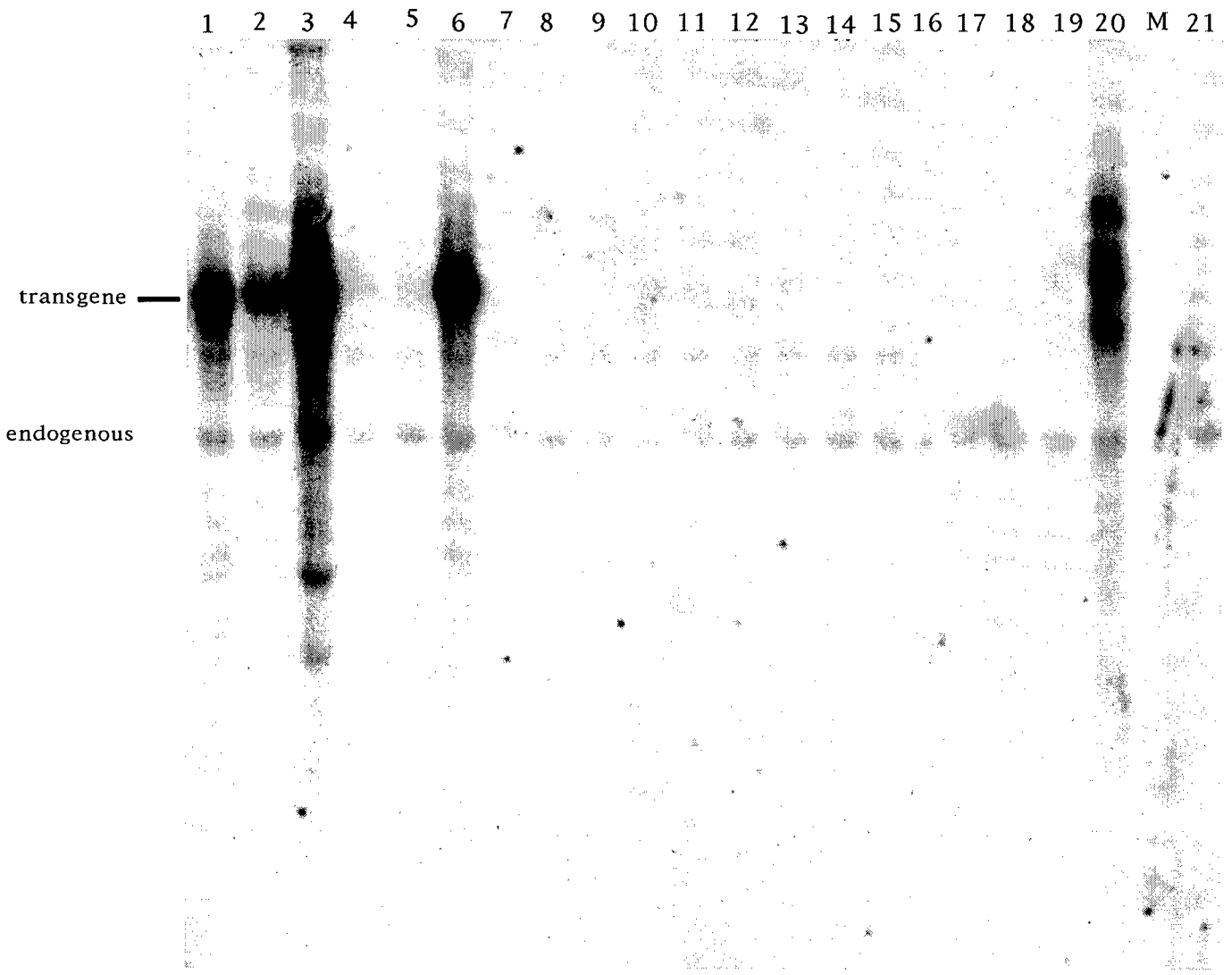

Figure 5

Southern blot analysis of transgenic founder mice carrying the 8 kb EcoRV frgment driving lacZ. The endogenous mouse hoxb-13 gene is indicated. The transgene is evident as a higher molecular weight hybridizing component in lanes 1,2,3,6, 19 and 20.

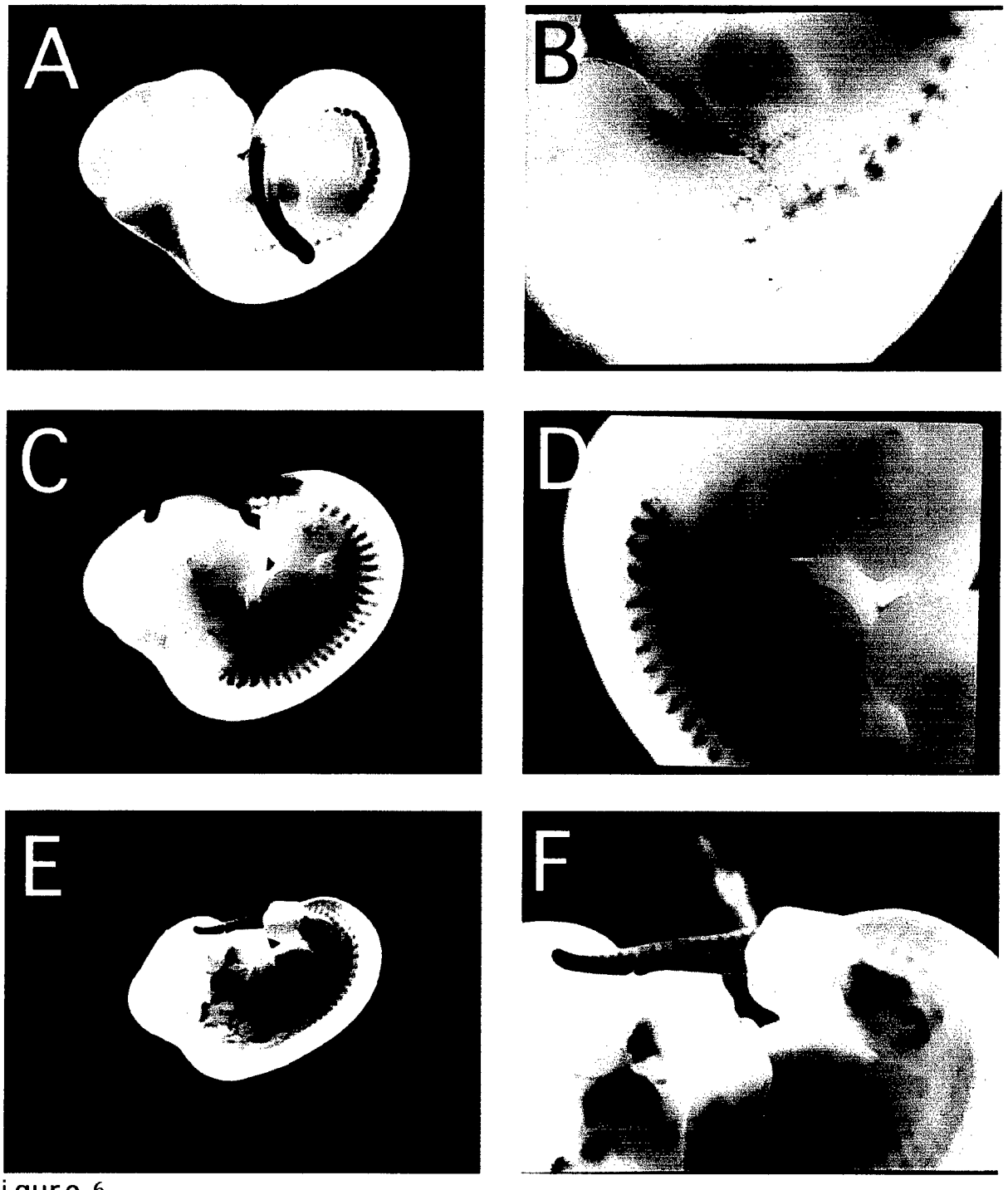

Figure 6
Embryos stained with X-gal at different stages of development. The blue indicates positive staining for lacZ activity. A)B13-6 embryo at 10.5days. B)Magnified view of A. Note the staining near the heart. C)B13-6 embryo at 11.5days. D)Magnified view of C. The ribcage and the spine are darkly stained. E)B13-2 embryo at 12.5days. F)Magnified view of E. Note the tail and the hindbimb staining, and the staining around the cranial area.

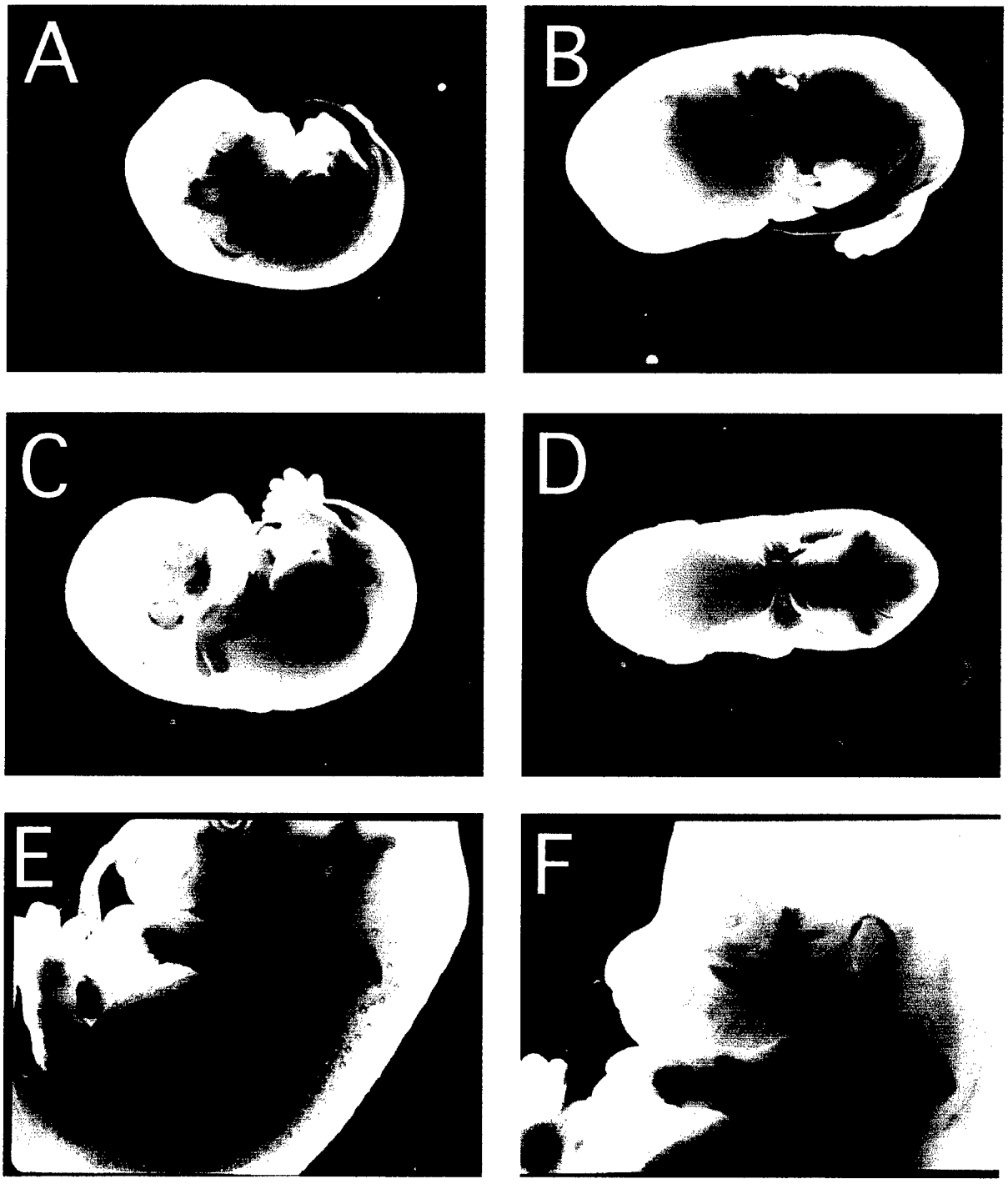

Figure 7
A)B13-2 embryo at 13.5days. B)Frontal view of B13-2 embryo at 13.5days. C)B13-20 embryo at 14.5days. D)Frontal view of the same embryo. E)B13-2 embryo at 14.5days. Note the nipple staining and staining in the cranial area. F)Magnified view of B13-6 at 14.5days. Note the cranial staining and the striated look of the staining of the forelimb.

KEY RESEARCH ACCOMPLISHMENTS

-generation of a physical map of a 50 kb region of the human *hoxb-13* locus

-generation of a physical map of a 50 kb region of the mouse *hoxb-13* locus

-identification of a PAC clone containing the human *hoxb-13* locus

-sublconing of genomic fragments upstream and downstream of *hoxb-13* loci

-generation of reporter gene constructs containing up to 8 kb of upstream DNA

-generation of transgenic mice carrying reporter constructs

- analysis of reporter gene expression in transgenic mice

REPORTABLE OUTCOMES

Manuscripts

Sreenath, T., Orosz, A., and Bieberich, C. J. (1999) Androgen-independent expression of *hoxb-13* in the mouse prostate. *The Prostate* 41, (in press).

Provisional Patent Application

Use of androgen-independent cis-acting DNA elements derived from Hox genes to direct therapeutic gene expression in human prostate cells. Charles J. Bieberich and Andras Orosz, inventors.

CONCLUSIONS

To date, we have succeeded in mapping a large region around both the human and mouse *hoxb-13* loci. Although significant difficulties were initially experienced, sublones carrying substantial portions of each locus have now been obtained. Several reporter genes carrying up to 8 kb of upstream DNA have been constructed, and transgenic mice carrying these have been generated. Analyses of reporter gene expression have revealed that the 8 kb fragment contains elements capable of directing a pattern of gene expression that closely mimics that of the endogenous hoxb-1`3 locus in early embryos. However, that fragment does not direct gene expression in the prostate gland in neonates or adults, which is a major site of expression of *hoxb-13* and the subject of investigation of this grant. In essence, we have established that the elements that direct *hoxb-13* expression in the prostate do not lie within 8 kb upstream of its translational start site. Our plan is to follow the

strategy outlined in the original application to widen our search for the prostate element by adding both farther upstream sequences as well as downstream sequences from the *hoxb-13* locus to our reporter constructs.

REFERENCES

none

APPENDIX 1

Galley Proofs

Androgen-independent expression of *hox b- 13* in the mouse prostate. *The Prostate* 41, (in press).

Rapid Communication

Androgen-Independent Expression of *hoxb-13* in the Mouse Prostate

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[2] *Department of Biological Sciences, University of Maryland, Baltimore County, Baltimore, Maryland*

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BACKGROUND. Hox genes encode transcriptional regulatory proteins that are largely responsible for establishing the body plan of all metazoan organisms. A subset of Hox genes is expressed during the period of organogenesis and into adulthood. *hoxb-13* is a recently-described member of the Hox gene family that is expressed in the spinal cord, hindgut, and urogenital sinus during embryogenesis.

METHODS. Northern blot and in situ hybridization analyses of *hoxb-13* expression in adult mouse tissues were performed.

RESULTS. *hoxb-13* mRNA is restricted to the prostate gland and distal colon in adult animals. In situ hybridization of mouse prostate tissue demonstrated that *hoxb-13* is expressed in the epithelial cells of the ventral, dorsal, lateral, and anterior prostate lobes. Accumulation of *hoxb-13* mRNA is not diminished following castration.

CONCLUSIONS. These data demonstrate that *hoxb-13* expression is androgen-independent in mouse prostate glands. The identification of *hoxb-13* as an androgen-independent gene expressed in adult mouse prostate epithelial cells provides a new potential target for developing therapeutics to treat advanced prostate cancer.

KEY WORDS: **homeobox gene; gene expression; castration; cancer**

INTRODUCTION

The mammalian Hox genes are homologs of *Drosophila* homeotic genes that encode homeodomain transcription factors [1]. Gain and loss of function analyses have demonstrated that Hox genes in mammals play a critical role in establishing the basic body plan, for example, by patterning the axial and the appendicular skeleton and parts of the central and peripheral nervous systems during embryogenesis [1–3]. An intriguing feature of some Hox genes is their continued expression during organogenesis and in differentiated organs in adult animals [1]. Although their roles in pattern formation during early embryonic development have been studied extensively, their roles in later developmental events and tissue maintenance have received comparatively little attention. The importance of understanding their functions in differentiated cells is underscored by the recognition that deregulated expression of Hox and other classes of homeobox-containing genes have been implicated in oncogenic transformation of cultured cells and in tumors [4–9].

Several members of the Hox family of homeobox genes have been found to be expressed in human prostate cell lines [6] and in mouse embryos in the region of the urogenital sinus that gives rise to pros-

Grant sponsor: U. S. Army Medical Research, Grant number: DAMD17-98-1-8477; Grant sponsor: Materiel Command Prostate Cancer Research Program.

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tate glands [10,11]. *hoxd-13* has been shown to be expressed in the urogenital sinus during late gestation and in early postnatal mouse prostate glands, but is repressed at maturity [10]. A loss-of-function mutation of *hoxd-13* in mice results in a decrease in size and ductal branching of the dorsal and ventral prostate lobes [12]. Both *hoxc-11* and *hoxb-13* have been reported to be expressed in the prostatic region of the urogenital sinus at 12.5 days gestation, but their distribution at later stages and in adult tissues have not been described [11,13].

We have characterized the distribution of *hoxb-13* mRNA in adult mouse tissues and have found that it is expressed at a high level in the prostate gland and distal colon.

MATERIALS AND METHODS

RNA extractions and Northern blot analyses were performed essentially as described [14] using either a 4.5 kb probe containing the entire *hoxb-13* coding region (T.S. and C.J.B., unpublished) or a 550 *Apa*I -*Eco*RI restriction fragment containing only exon 1. Identical results were obtained with both probes. Dissection of prostate glands into component lobes was performed as described [15]. Orchiectomy was carried out on 6-week-old CD-1 mice as described for rats [16], and RNA was extracted from whole prostate glands pooled from two mice at each time point. In situ hybridization was performed as described [17] using 0.45 kb *Bgl*II-*Xba*I fragment derived from the 3′ end of *hoxb-13* [13].

RESULTS AND DISCUSSION

To determine whether *hoxb-13* is expressed in adult tissues, Northern blot analyses were performed. A 4.5 kb probe derived from the *hoxb-13* genomic locus and containing the entire transcription unit was hybridized to total RNA extracted from 18 different tissues of adult FVB mice (Fig. 1 and data not shown). A strongly hybridizing 3.2 kb component was observed only in RNA from the prostate and the distal region of the colon (Fig. 1A,B). Within the prostate gland, *hoxb-13* mRNA was detected in all lobes (Fig. 1A). When normalized to the level of a housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), the ventral and lateral lobes showed a similar steady-state level of expression, while in the coagulating gland (anterior prostate) and dorsal prostate the level was consistently 2-3 fold lower. A distal-to-proximal gradient of *hoxb-13* mRNA was observed in the colon (Fig. 1B). The rectum and 1 cm of adjacent colon showed the highest level of expression, while in the most proximal 1 cm of colon, just distal to the cecum,

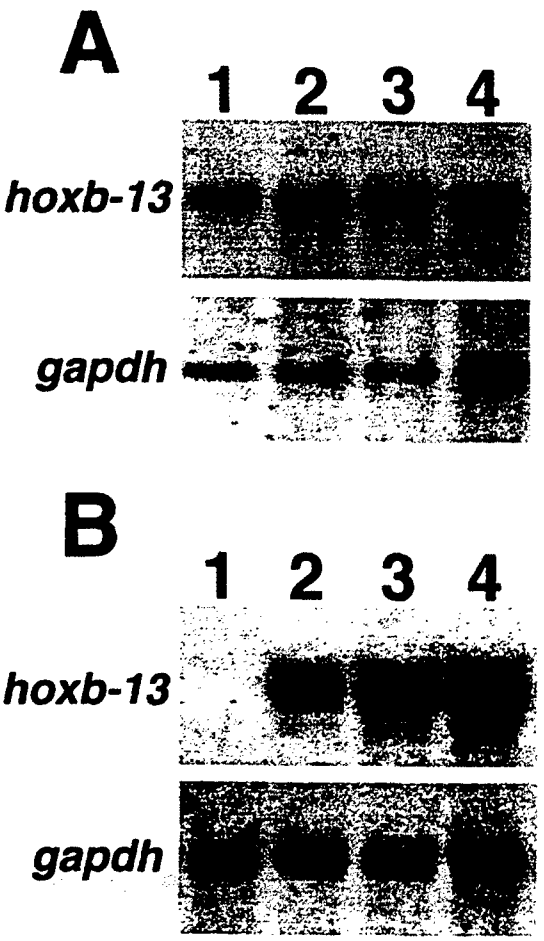

Fig. 1. Northern blot analysis of *hoxb-13*m RNA expression in adult mouse tissues. Panel **A**: lane 1, coagulating gland; lane 2, ventral prostate; lane 3, lateral prostate; lane 4, dorsal prostate. Panel **B**: lane 1, proximal colon, defined as 1 cm of intestinal tissue just distal to the cecum; lane 2, middle colon, defined as the central 1 cm of tissue between the distal margin of the cecum and the anus; lane 3, distal colon, defined as 1cm of colon adjacent to the rectum; lane 4, rectum. Average length of colon from the distal margin of the cecum to the anus was 8 cm.

hoxb-13 mRNA was not detected (Fig. 1B). *hoxb-13* mRNA was also detected in the central region of the colon at a level two-fold lower than in the rectum (Fig. 1B). Expression of *hoxb-13* was not detected by Northern blot analysis in other urogenital tissues including kidney, testis, urethra, bladder, seminal vesicle, ampullary gland, vas deferens, ovary, or uterus (data not shown). *hoxb-13* expression was also not detected in RNA from preputial gland, liver, spleen, lung, heart, thymus and brain (data not shown).

To characterize the cellular distribution of *hoxb-13* mRNA within the mouse prostate gland, in situ hybridization analyses were performed on histological sections of adult tissue. As predicted by the Northern blot analyses, hybridization to the *hoxb-13* probe was observed in all prostate lobes (Fig. 2 and data not

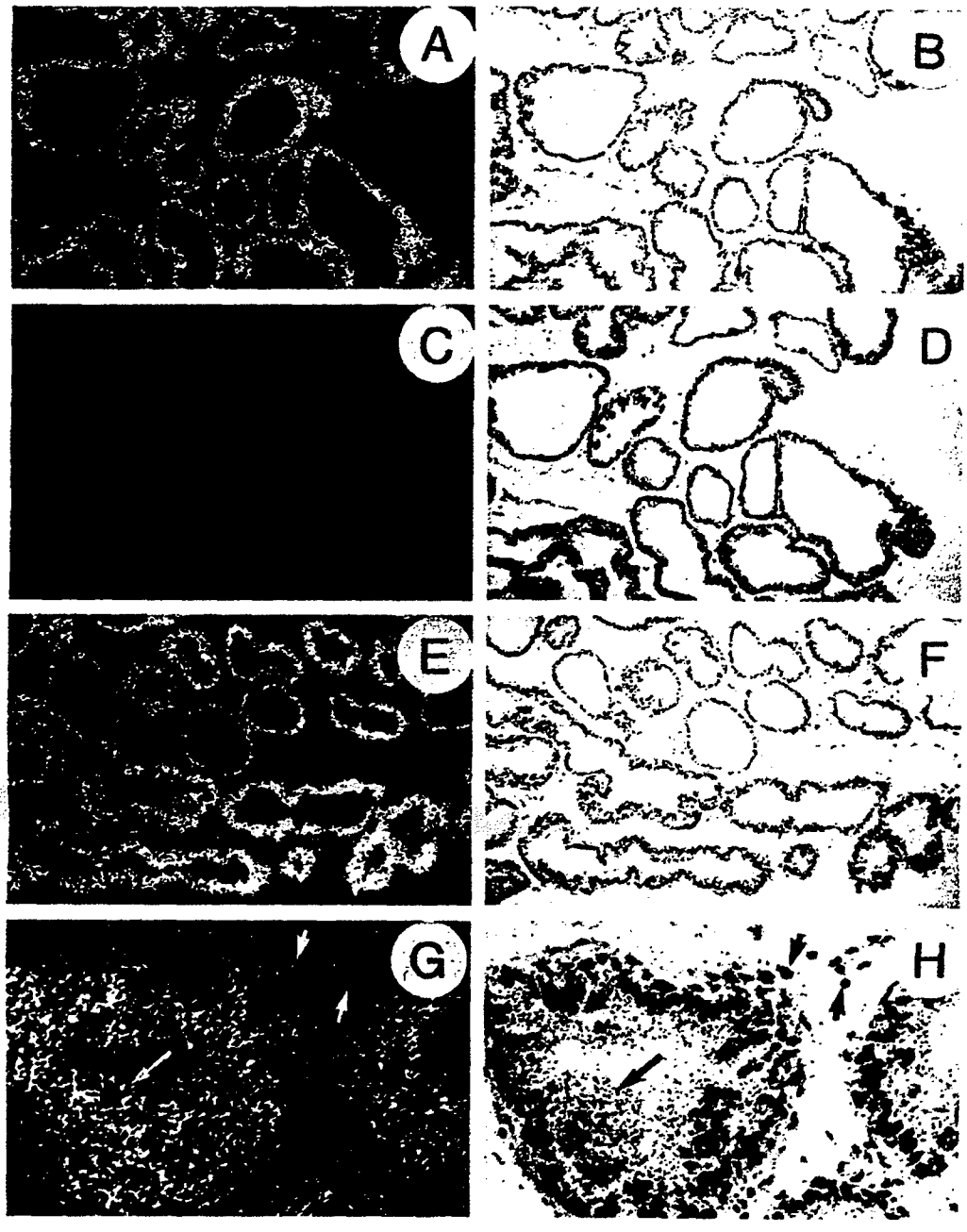

Fig. 2. In situ hybridization analysis of *hoxb-13* expression in adult prostate. Paraffin sections of ventral (A-D) and lateral (E-H) lobes. **A**, darkfield, **B**, brightfield low magnification view shows signal over ducts in the ventral lobe using the antisense probe. **C**, darkfield, **D**, brightfield view shows lack of signal with a sense probe on a serial section, demonstrating the specificity of the assay. **E**, darkfield, **F**, brightfield low magnification view of lateral lobe ducts. **G**, darkfield, **H**, brightfield high magnification view of a lateral duct shows hybridization signal over epithelial cells. Short arrows highlight stromal cells, long arrow points to epithelial cells.

shown). Hybridization signal was seen along the length of the ducts but was excluded from the proximal region of each main duct near the junction with the urethra. Within the ducts, strong hybridization signal was observed over luminal epithelial cells, while stromal cells did not display signal that was distinguishable from background.

The effect of castration-induced androgen deprivation on the steady-state level of *hoxb-13* mRNA was examined by Northern blot analysis. Surprisingly, the level of *hoxb-13* mRNA was not diminished within 8 days after orchiectomy when normalized to the level of GAPDH (Fig. 3 and data not shown). In contrast, expression of *mp12*, an androgen-dependent gene which encodes a protease inhibitor, decreased 70-fold 24 hours after castration and was not detectable after

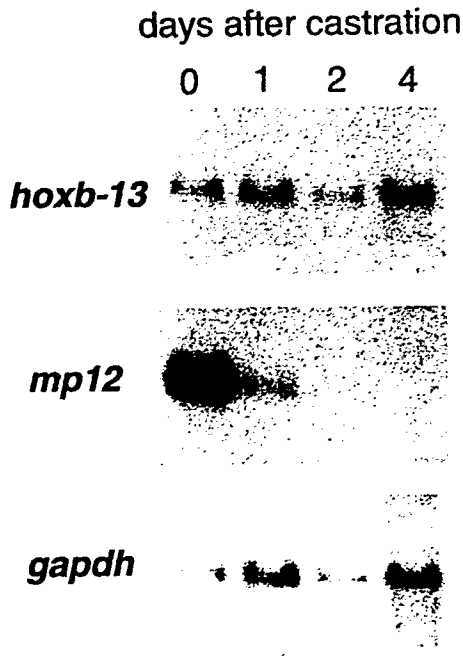

Fig. 3. Northern blot analysis of *hoxb-13* expression in orchiectomized mice. The same Northern blot membrane was sequentially hybridized with the indicated probes.

48 hours (Fig. 3) [18]. Similarly, the level of mRNA encoded by *nkx-3.1*, a homeobox gene that has been demonstrated to be androgen-dependent, was decreased nearly 10-fold within 24 hours, and 30-fold after 4 days (data not shown). These data indicate that maintenance of a high steady-state level of *hoxb-13* mRNA in the prostate gland does not require testicular androgens. It has been demonstrated in rats that a significant proportion (~20%) of ventral prostate epithelial cells survive at least 1 week after castration [19]. Our observations suggest that *hoxb-13* expression persists, and may even be up-regulated in the mouse prostatic epithelial cells that survive after castration. Despite the fact that it is the most diseased organ in the human body, the genetic basis of prostate development, differentiation, and maintenance is not well understood. Considerable effort is currently focused on defining the role of androgens and peptide growth factors in prostate development and disease [20]. However, the nature of the genetic program(s) active in epithelial cells that underlie differentiation and maintenance remain largely undefined. Recently, several homeobox genes have been strongly implicated in both normal and malignant growth of prostate epithelial cells. The mouse *nkx-3.1* homeobox gene has been found to be expressed in developing and mature prostate glands where it is restricted to ductal epithelial cells and is androgen-dependent [14,21,22]. *nkx-3.1* is the earliest known marker of prostatic epithelial cells, and a loss-of-function mutation results in reduced ductal branching in all prostate lobes as well as defects in secretory protein production [23]. *nkx-3.1* null mice also develop epithelial hyperplasia and dysplasia in the anterior and dorsolateral lobes that increases in severity with age, suggesting that this homeobox gene may be a candidate tumor suppressor gene [23]. In contrast, the homeobox genes GBX1 and GBX2 have been found to be overexpressed in prostate cancer cell lines, and reduction of GBX 2 expression results in decreased clonogenic ability in vitro and tumorigenicity in vivo [6]. Together these data suggest that both gain and loss of function of certain homeobox genes may play a role in prostate cancer progression. Interestingly, human *hoxb-13* expressed sequence tags have been observed in both prostate and colon carcinomas [24].

Although most prostate tumors initially respond to androgen deprivation therapy (ADT), nearly all return as androgen-independent disease. Currently, there is a dearth of effective treatment options available to patients with advanced prostate cancer. The need to identify new potential targets for therapeutic intervention and to develop novel therapies to treat androgen-independent prostate cancer is axiomatic. Our observation of androgen-independent expression of *hoxb-13* in the mouse prostate provides a new model to study the molecular basis of androgen-independent gene expression in prostate epithelial cells.

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24. The Unigene System at www.ncbi.nlm.nih.gov/UniGene/index.html was used to search Genbank for human *hoxb-13* expressed sequence tags.

LEAVE AS REF

APPENDIX 2

Invention disclosure statement

Use of androgen-independent cis-acting DNA elements derived from Hox genes to direct therapeutic gene expression in human prostate cells. Charles J. Bieberich and Andras Orosz, inventors.

I. DISCLOSURE OF INVENTION

1. Title of Invention

Use of androgen-independent cis-acting DNA elements derived from Hox genes to direct therapeutic gene expression in human prostate cells

2. List of Inventors:

	Name	Title	Department	Telephone
A.	Charles J. Bieberich	Ph. D.	Biological Sciences	3125
B.	Andras Orosz	Ph.D.	Biological Sciences	2629

3. Ownership. In my opinion this invention is:

A. Owned by UMAB/UMBC in accordance with the University of Maryland System Patent Policy.

B. Was developed by the inventor(s) without use of UMAB/UMBC time, facilities or materials and belongs to the inventor(s).

C. Is subject to special waiver in accordance with the University of Maryland System Patent Policy.

4. Advisor approval for student submissions (if applicable):

Name/Title: _____

Date: _____

5. Description of Invention.

A) In your own words, how would you briefly describe the invention?

The invention consists of a novel use for gene promoters/enhancers derived from Hox genes. Some Hox genes are expressed in prostate cells, and some of these are expressed in an androgen-independent manner. This invention consists of using cis-acting DNA elements derived from androgen-independent Hox genes to direct gene expression in human prostate cells.

B) Is the invention a new process, composition of matter, a device or one or more products? A new use for, or an improvement to, an existing product or process?

product

NOTE: **use additional sheets if necessary. You may also attach a manuscript, research proposal, drawing, or any other material that would assist in understanding the invention.**

6. **Novel Features.** Pick out and expand on the novel and unusual features of the invention. How does it differ from present technology? What problems does it solve? What advantages does it possess?

Hox gene cis-acting elements are the only known promoters/enhancers that are preferentially expressed in adult human prostate cells in an androgen-independent manner. This feature makes them particularly well-suited to be used in gene therapy approaches to treat advanced prostate cancer. However, they will also be useful in treating earlier stages of prostate cancer.

7. **Uses and Applications.** What are the possible uses for the invention? What products could be developed? In addition to immediate applications, are there other uses that might be realized in the future?

The main use of this invention will be in gene therapy treatments for prostate cancer. Currently, all known promoter/enhancers that are primarily active in prostate cells require testosterone for their activity. Since advanced prostate cancer patients typically do not have androgens (that is, they have been castrated) these promoter/enhancers are the only known DNA elements suitable for use in gene therapy vectors to treat advanced prostate cancer in patients that have undergone androgen deprivation therapy.

8. Reduction to Practice.

A) When was the invention first conceived? October 1997

Is this date documented? NO Where?

B) Has the invention been tested experimentally? no. Please state if you have preliminary results, animal or laboratory models, prototypes, clinical tests, etc.
(Simply reference appropriate sections if you have already included this information.)

Preliminary experiments indicate that both the mouse and human *hoxb-13* genes are expressed in prostate cells in an androgen independent manner. However, since Hox genes are part of a mutitgene family and are among the most conserved genes known, it is likely that other Hox genes from many species will also be expressed in prostate cells in an androgen-independent manner. In fact, the cis-acting elements that cause these genes to be expressed in prostate cells in this fashion may be present in mutiple Hox genes and in multiple species. We have isolated a DNA region from the human *hoxb-13* gene that we believe directs gene expression to prostate cells.

9. **Obstacles.** Does the invention have any disadvantages or limitations? How can they be overcome?

no

Disclosure No. _____

II. SUPPORTING INFORMATION

1. Publications. Has the invention or a similar invention in whole or in part been described in a publication? (A Publication@ for this purpose includes abstracts of talks, new stories, scientific papers, thesis, etc.) Has the invention been described orally at meetings? **Please provide exact details including dates an copies of any publications.**

No

2. Disclosure.

A) Has this invention been disclosed other than as described in 1.? If so, please specify the date, place and circumstances. If disclosed to specific individuals, please give names and dates.

This idea has been described in the attached grant proposal submitted to the United States Army Medical Research and Materiel Command Prostate Cancer Research Program on October 28,1997.

B) Are you planning any disclosure of the invention within the next six months? Is there an imminent publication, oral presentation, showing or sale of the invention? Please give best estimate of dates and locations.

The abstract of the Army grant proposal will be posted on the World Wide Web sometime after 1 Oct 98.

3. Prior Art.

A) A literature search should be done by the inventor to determine publications relevant to this invention. Please list publications and any related patents you may know. Use

additional sheets if necessary.

Zeltser L; Desplan C; Heintz N., Howard Hughes Medical Institute, Rockefeller University, New York, NY 10021, USA. Development 1996 Aug;122(8):2475-84.

B) Do you know of relevant information presented at a public talk, trade fair, sales catalogue? Has the invention or a similar product been used publicly or has it been offered for sale?

no

4. Sponsorship.

Who sponsored or paid for the work that led to the invention or part thereof? (Federal or State agency, industry, DRIF, UMAB/UMBC, etc.)

SPONSOR	CONTRACT OR GRANT NO.
NIH	R29 HD27943

5. Marketing Information.

A) Has there been any commercial interest in this invention? Please name companies and specific persons, if known.

no

B) What firms do you think may be interested in this invention? Why?

Pharmaceutical companies interested in gene therapy for prostate cancer. This is the only known DNA element capable of directing gene expression in prostate cells in an androgen independent manner. Since advanced prostate cancer patients typically do not have androgens (that is, they have been castrated) this promoter is the only one suitable for use in gene therapy vectors to treat this disease.

C) Are there any products currently in the market that compete with your invention? What company(ies) makes them?

no

D) Can you help us estimate the potential size of the market? For example, if the invention is a new therapeutic agent, can you give an estimate of the number of people afflicted in the U.S. and abroad?

I don't know the number of advanced prostate cancer patients in the US annually, but nearly 40,000 men die each year in the US from prostate cancer. Presumably, many of these would be good candidates for gene therapy.

E) Would your invention be primarily used in countries other than the U.S.? (e.g. the invention is a vaccine for a tropical disease, etc.)

no

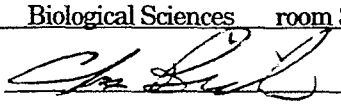
F) Do you know of other research groups that may be working on similar inventions? Who are they and where are they located?

Don't know

6. Inventor(s) Information and Signature.

Name: Charles J. Bieberich Telephone: 3125

Address: Biological Sciences room 323

Signature:  Date: 6 AUG-98

Name: Andras Orosz Telephone: 2629
Address: Biological Sciences room 323

Signature: [signature] Date: 8/6/1998

Name: _____ Telephone: _____
Address: _____

Signature: _____ Date: _____

Witness:

[signature] 8/6/1998
Signature Date

Audrey J. Ellis
Typed Name

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
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REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 1 Apr 03

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession document numbers be changed to "Approved for public release; distribution unlimited." Copies of these reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLLIS M. RINEHART
Deputy Chief of Staff for
Information Management

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